

(1) A rapid, whole-tissue assay of PS II in leaves



Pasquale Losciale^{1,2}, Riichi Oguchi^{1,3}, Luke Hendrickson^{1,4},
Alexander B. Hope⁵, Luca Corelli-Grappadelli², and

Wah Soon Chow¹

Fred. Chow@anu.edu.au

¹ Research School of Biological Sciences and ⁴ ARC Centre of Excellence in Plant Energy Biology, Australian National University, Australia

² Dipartimento Colture Arboree, University of Bologna, via Fanin 46, 40127 Bologna, Italy

³ Plant Sciences, Department of Biological Sciences, Graduate School of Science, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan

⁵ School of Biological Sciences, Flinders University, GPO Box 2100, SA 5001, Australia

(2) Aims:

- ♣ To devise a rapid, whole-tissue assay of the functional fraction of Photosystem II after progressive photoinactivation;
- ♣ To test the universality of the correlation of the assay with the actual fraction of functional PS II in C3 and C4, herbaceous and woody, wild type and Chl *b*-less mutant, and monocot and dicot plants.

Introduction:

- ♣ The O₂/flash directly measures active PS II, but is time-consuming.
- ♣ Chl fluorescence only represents chloroplasts near leaf surface.
- ∴ We devised a rapid, whole-tissue assay of PS II after photoinhibition.

(3) Methods

(a) Continuous far-red (FR)

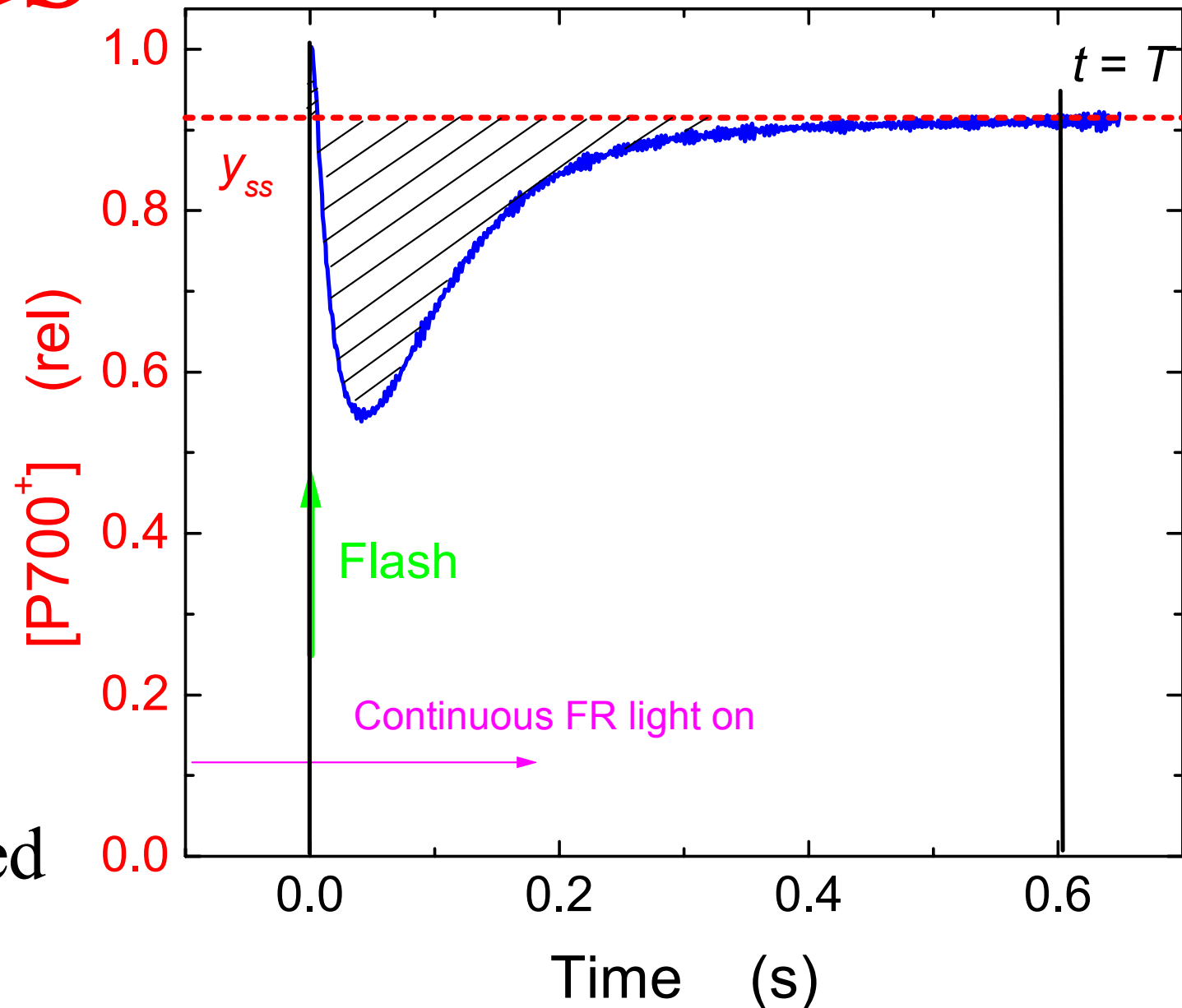
on: $[P700^+] = y_{ss}$

(b) Single turnover flash

at $t = 0$

(c) Electrons arrive from PS II,
while FR re-oxidizes P700

(d) The integrated, flash-induced
electron flow to $P700^+$ is

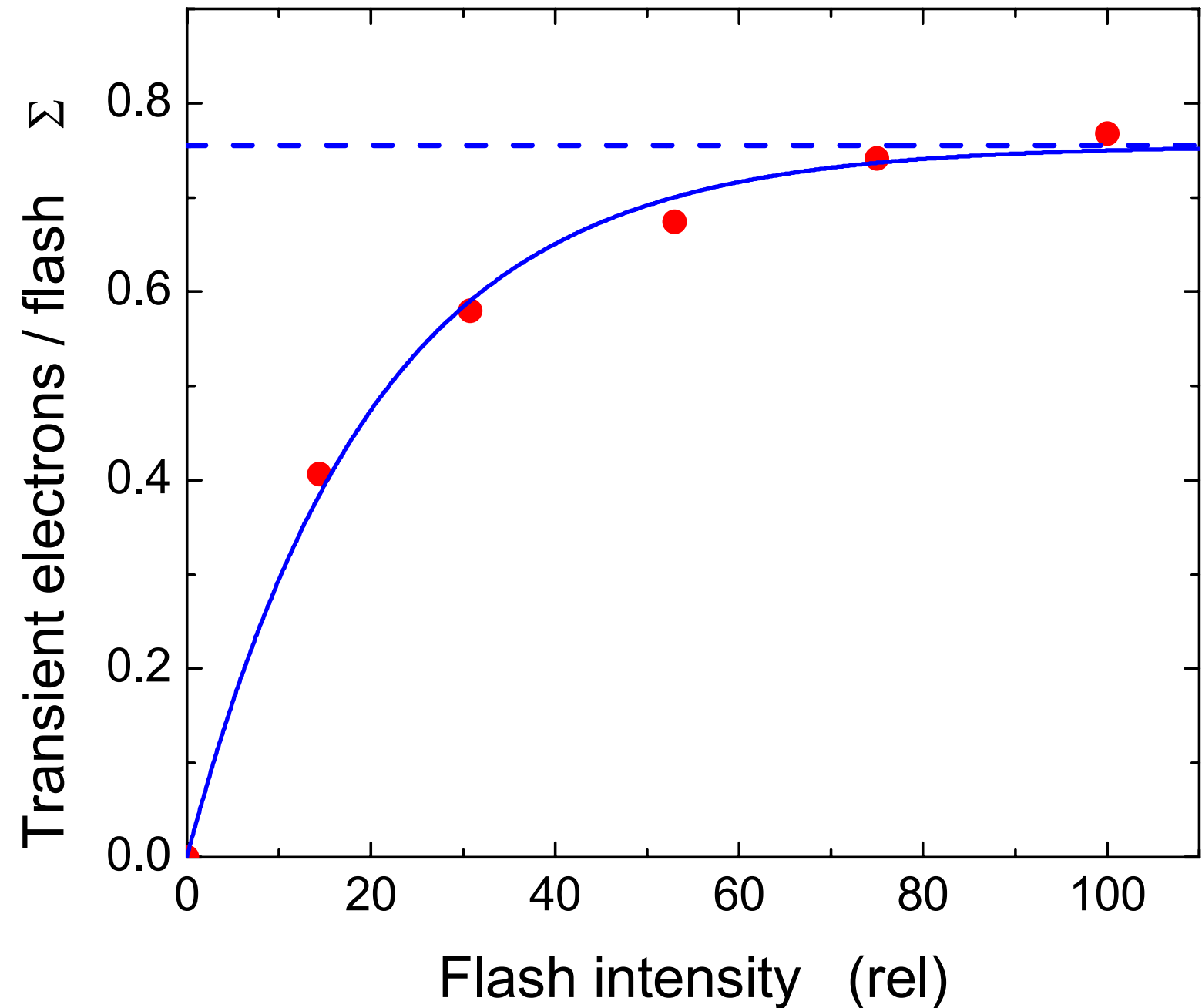


$$\Sigma = (1 - y_{ss}) + k_o \int_0^T (y_{ss} - y) dt \quad (k_o = \text{rate coefficient of photo-oxidation; integral is given by the shaded algebraic area})$$

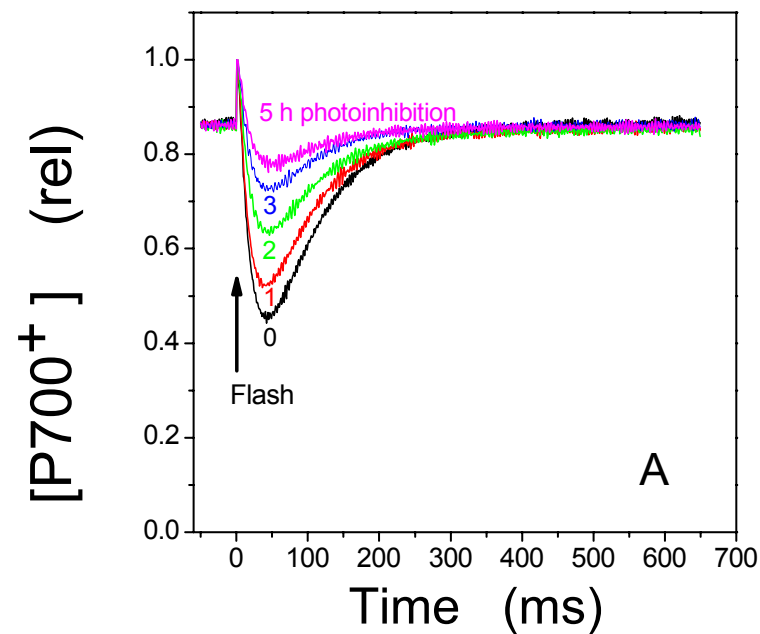
(4) Results

The integrated, transient electron flow to $P700^+$ was saturated at the max flash intensity.

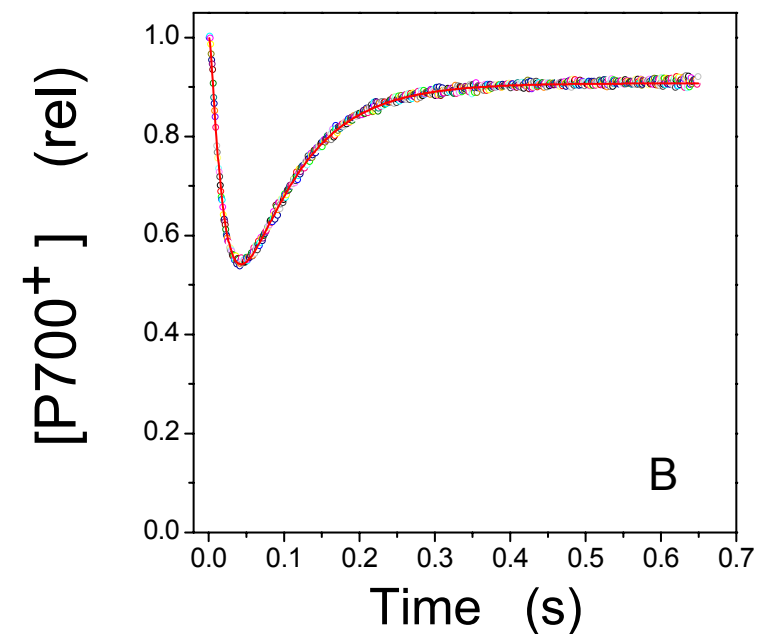
i.e., all active PS II units in the whole tissue were excited by the flash.



(5) Transient electrons per flash (Σ) decreased with photoinhibition

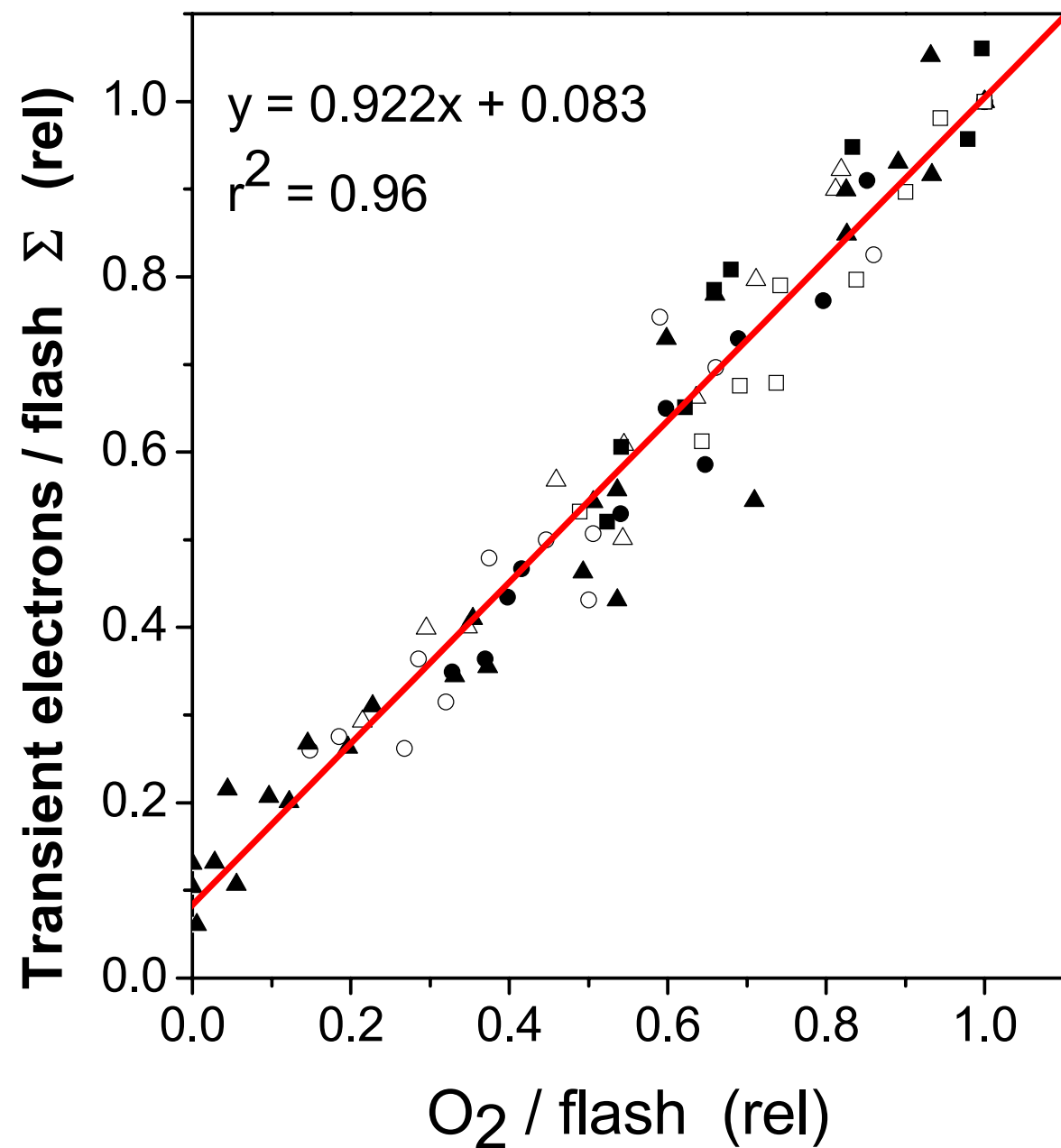


With increasing duration of photoinhibition in the presence of lincomycin, the dip became shallower, i.e., the transient electrons per flash (Σ) is decreased.



Curve fitting of data points yielded k_o (the rate coefficient of P700 photo-oxidation) that is needed to evaluate Σ .

(6) Linear correlation of Σ with O_2 /flash for all plants tested



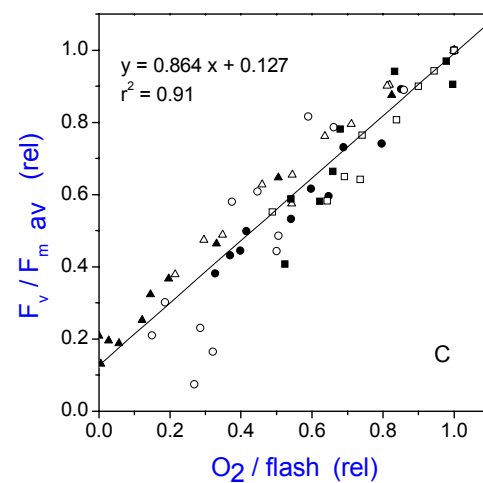
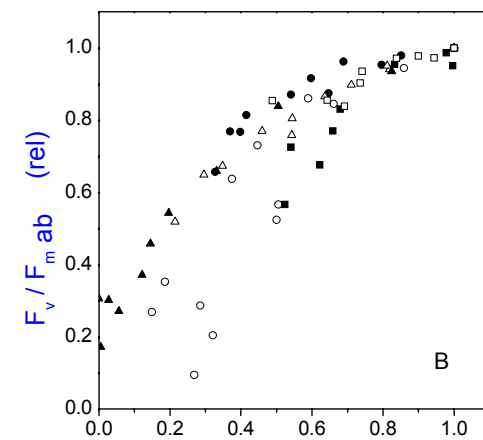
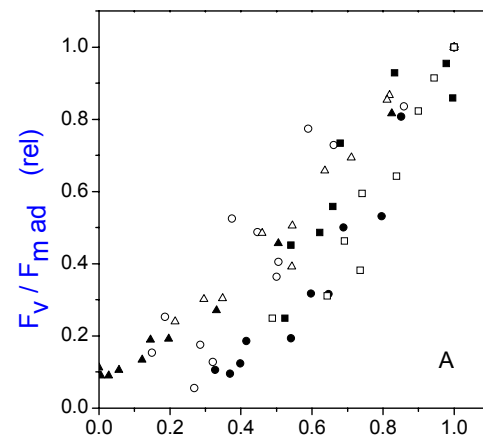
With increasing duration of photoinhibition, Σ decreased linearly with O_2 /flash.

The same linear correlation holds for C3 and C4, woody and herbaceous, wild type and Chl *b*-less mutant, and monocot and dicot plants.

Nectarine (●), *Arabidopsis* (○), wild type barley (△), Chl *b*-less barley (■), capsicum (▲), and *Flaveria bidentis*, a C4 species (□).

(7)

Chl fluorescence F_v/F_m is less well correlated



F_v/F_m measured on the adaxial surface showed large scatter, the correlation tending to curve downwards.

F_v/F_m measured on the abaxial surface also showed large scatter, the correlation tending to curve upwards.

The average F_v/F_m for both surfaces showed an improved correlation, but with some outlying points. Symbols as for Panel 6.

(8) Discussion:

- ♣ Measurement of Σ involves the whole tissue, in contrast to that of F_v/F_m ;
- ♣ This helps to explain the seemingly universal the correlation of Σ with the actual fraction of functional PS II in C3 & C4, herbaceous & woody, wild type & Chl *b*-less mutant, and monocot & dicot plants.
- ♣ Measurement of Σ is non-intrusive, rapid and applicable to leaves attached to the plant.
- ♣ It is potentially applicable in the field.